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### Preperoxisomal vesicles can form in the absence of Pex3

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*Published in:*  
The Journal of Cell Biology

*DOI:*  
[10.1083/jcb.201310148](https://doi.org/10.1083/jcb.201310148)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2014

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Knoops, K., Manivannan, S., Cepinska, M. N., Krikken, A. M., Kram, A. M., Veenhuis, M., & van der Klei, I. J. (2014). Preperoxisomal vesicles can form in the absence of Pex3. *The Journal of Cell Biology*, 204(5), 659-668. <https://doi.org/10.1083/jcb.201310148>

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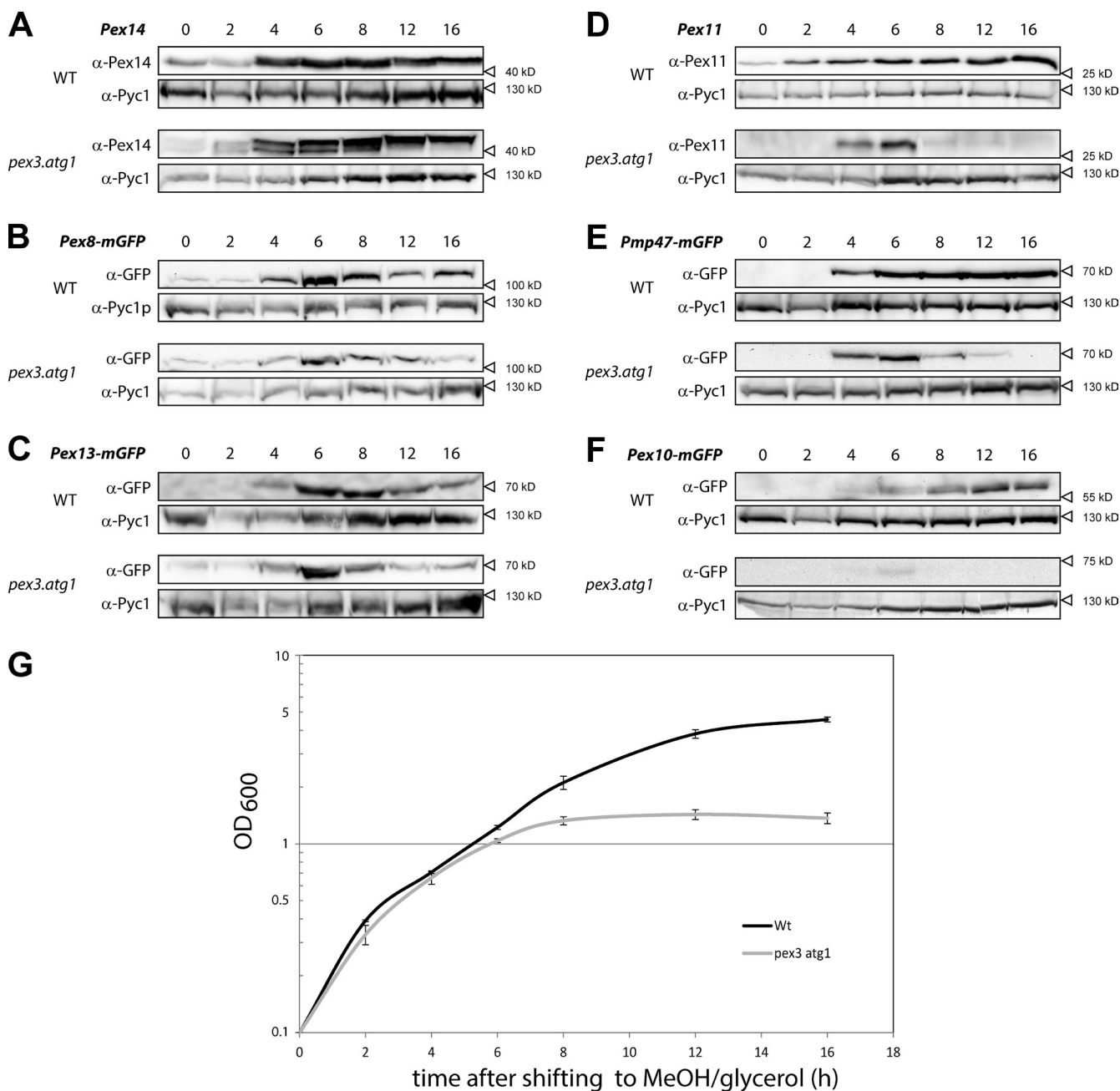
Knoops et al., <http://www.jcb.org/cgi/content/full/jcb.201310148/DC1>

Figure S1. **Induction of PMPs in WT and *pex3 atg1* cells.** (A–F) MM-Glu grown cells were shifted to MM-M/G. Samples were taken at the indicated time points. (G) Corresponding growth curves. Error bars indicate SD of five experiments.

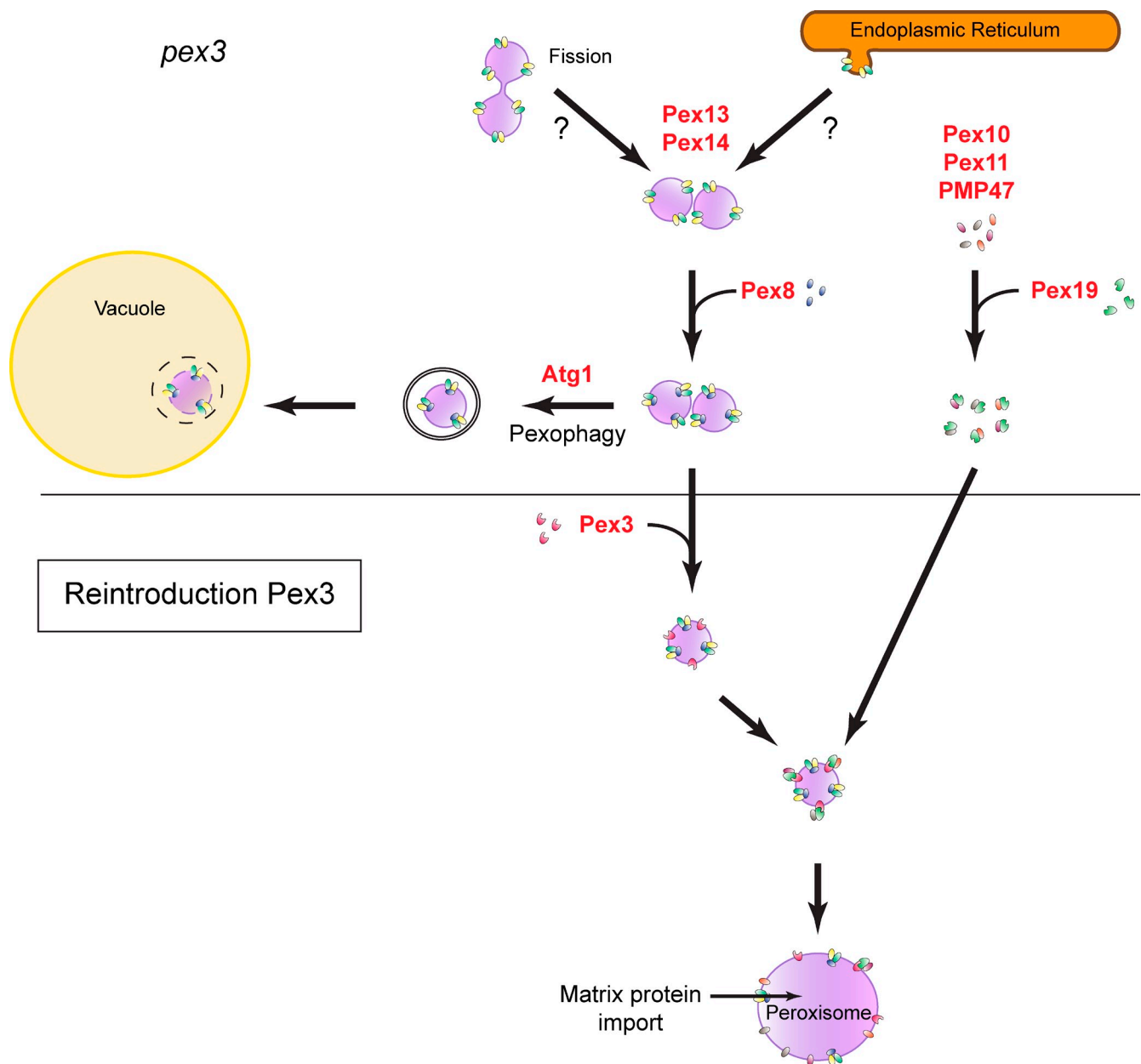


Figure S2. **Model.** In *pex3* cells, Pex13/Pex14-containing structures exist that import Pex8. These structures may arise by proliferation of a preexisting structure or form from the ER. They are constitutively degraded by autophagy, unless *ATG1* is deleted. Pex10, Pex11, and PMP47 are unstable, because they are not inserted into these structures. Reintroduced Pex3 is sorted to the structures, followed by insertion of the other PMPs by the Pex3–Pex19 machinery and import of matrix proteins.

Table S1. *H. polymorpha* strains used in this study

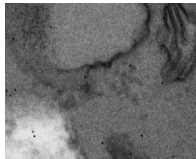
Strains	Characteristics	Reference
WT	NCYC 495 <i>leu1.1</i>	Saraya et al., 2012
<i>atg1</i>	ATG1 deletion strain, <i>leu1.1</i>	Komduur et al., 2003
<i>pex3</i>	PEX3 deletion strain, <i>ura3</i>	Baerends et al., 1996
<i>pex19</i>	PEX19 deletion strain, <i>ura3</i>	Otzen et al., 2004
<i>pex10</i>	PEX10 deletion strain	Tan et al., 1995
<i>pex3 atg1</i>	PEX3 ATG1 double deletion strain	This study
<i>pex19 atg1</i>	PEX19 ATG1 double deletion strain	This study
<i>pex3 atg1.PEX14-mCherry</i>	<i>pex3 atg1</i> with pSEM01	This study
<i>pex3 atg1.PEX14-mCherry.PEX8-mGFP</i>	<i>pex3 atg1</i> with pSEM01 and pMCE4	This study
<i>pex3 atg1.PEX14-mCherry.PEX10-mGFP</i>	<i>pex3 atg1</i> with pSEM01 and pMCE5	This study
<i>pex3 atg1.PEX14-mCherry.PEX11-mGFP</i>	<i>pex3 atg1</i> with pSEM01 and pSEM02	This study
<i>pex3 atg1.PEX14-mCherry.PEX13-mGFP</i>	<i>pex3 atg1</i> with pSEM01 and pSEM03	This study
<i>pex3 atg1.PEX14-mCherry.PMP47-mGFP</i>	<i>pex3 atg1</i> with pSEM01 and pMCE7	This study
<i>pex3 atg1.PEX13-mGFP</i>	<i>pex3 atg1</i> with pSEM03	This study
<i>pex3 atg1.PEX14-mCherry-BiP<sub>N30</sub>-eGFP-HDEL</i>	<i>pex3 atg1</i> with pSEM01 and pRSA017	This study
<i>pex3 atg1.PEX14-mCherry.PEX10-mGFP – P<sub>AOX</sub> PEX19</i>	<i>pex3 atg1</i> with pSEM01, pMCE05 and pSEM05	This study
<i>pex3 atg1.PEX14-mCherry.PMP47-mGFP – P<sub>AOX</sub> PEX19</i>	<i>pex3 atg1</i> with pSEM01, pMCE07 and pSEM05	This study
<i>pex3 atg1.PEX14-mGFP</i>	<i>pex3 atg1</i> with pSNA12	This study
<i>pex3 atg1 pex25</i>	<i>pex3 atg1</i> with pRSA018	This study
<i>pex3 atg1 pex25.PEX14-mGFP</i>	<i>pex3 atg1 pex25</i> with pSNA12	This study
<i>pex3 atg1.PEX14-mCherry – P<sub>AMO</sub> PEX3-eGFP</i>	<i>pex3 atg1</i> with pSEM01 and pHIPZ5-P <sub>AMO</sub> PEX3-eGFP	This study
<i>pex3 atg1.PEX14-mCherry.PEX10-mGFP-P<sub>AMO</sub> PEX3</i>	<i>pex3 atg1</i> with pSEM01, pMCE5 and pSEM04	This study
<i>pex3 atg1.PEX14-mCherry.PMP47-mGFP-P<sub>AMO</sub> PEX3</i>	<i>pex3 atg1</i> with pSEM01, pMCE7 and pSEM04	This study
<i>pex3 atg1.PEX14-mCherry – P<sub>AMO</sub> PEX3-eGFP – P<sub>TEF1</sub> eGFP-SKL</i>	<i>pex3 atg1</i> with pSEM01, pSEM04 and pAKW27	This study
<i>pex10. PEX8-mGFP.PEX14-mCherry</i>	<i>pex10</i> with pSEM01 and pMCE4	This study
WT. <i>PEX13-mGFP</i>	WT with pSEM03	This study
WT. DsRed-SKL. <i>PEX8-mGFP</i>	WT. DsRed-SKL with pMCE4	Cepińska et al., 2011
WT. DsRed-SKL. <i>PEX10-mGFP</i>	WT. DsRed-SKL with pMCE5	Cepińska et al., 2011
WT. DsRed-SKL. <i>PMP47-mGFP</i>	WT. DsRed-SKL with pMCE7	Cepińska et al., 2011
WT. <i>PEX10-mGFP.PEX14-mCherry</i>	WT with pSEM01 and pMCE7	This study
WT. <i>PEX11-mGFP.PEX14-mCherry</i>	WT with pSEM01 and pMCE3	This study
WT. DsRed-SKL. <i>PEX13-mGFP</i>	WT. DsRed-SKL with pSEM03	This study

Table S2. Plasmids used in this study

Plasmid	Characteristics	Reference
pSEM01	pHIPN plasmid containing C-terminal part of <i>PEX14</i> fused to mCherry; <i>nat<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	This study
pSEM02	pHIPZ plasmid containing C-terminal part of <i>PEX11</i> fused to mGFP; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	This study
pSEM03	pHIPZ plasmid containing C-terminal part of <i>PEX13</i> fused to mGFP; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	This study
pSEM04	pHIPH5 plasmid containing <i>PEX3</i> under control of P <sub>AMO</sub> , <i>Hph<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	This study
pSEM05	pHIPH4 plasmid containing <i>PEX19</i> under control of P <sub>AOX</sub> , <i>Hph<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	This study
pSEM188	pBS plasmid containing <i>PEX19</i> deletion cassette; <i>LEU2</i> ; <i>amp<sup>R</sup></i>	This study
pHIPZ5-PEX3-eGFP	pHIPZ5 plasmid containing <i>PEX3</i> -eGFP under control of P <sub>AMO</sub> , <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Nagotu et al., 2008
pRSA017	pHIPZ4 plasmid containing BiP <sub>N30</sub> -eGFP-HDEL under control of P <sub>AOX</sub> , <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Saraya et al., 2011
pHIPH4	pHIPH plasmid containing hygromycin B marker, <i>amp<sup>R</sup></i>	Saraya et al., 2011
pHIPZ7	pHIPZ plasmid containing TEF1 promoter, <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Baerends et al., 1997
pFEM35	pHIPX7 plasmid containing eGFP with C-terminal PTS1 under control of P <sub>AOX</sub> , <i>Leu2</i> ; <i>kan<sup>R</sup></i>	Krikken et al., 2009
pHIPZ-mGFP fusinator	pHIPZ plasmid containing mGFP and AMO terminator; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Saraya et al., 2010
pMCE02	pHIPN plasmid containing mCherry; <i>nat<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Cepińska et al., 2011
pSNA12	pHIPZ plasmid containing C-terminal part of <i>PEX14</i> fused to mGFP; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Cepińska et al., 2011
pMCE3	pHIPN pPlasmid containing C-terminal part of <i>PEX11</i> fused to mCherry; <i>nat<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Cepińska et al., 2011
pMCE4	pHIPZ plasmid containing C-terminal part of <i>PEX8</i> fused to mGFP; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Cepińska et al., 2011
pRSA018	pDEST-R4-R3 containing <i>PEX25</i> deletion cassette, <i>nat<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Saraya et al., 2011
pMCE5	pHIPZ plasmid containing C-terminal part of <i>PEX10</i> fused to GFP; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Cepińska et al., 2011
pMCE7	pHIPZ plasmid containing C-terminal part of <i>PMP47</i> fused to GFP; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	Cepińska et al., 2011
pAKW27	pHIPZ plasmid containing eGFP-SKL under control of P <sub>TEF1</sub> ; <i>zeo<sup>R</sup></i> ; <i>amp<sup>R</sup></i>	This study
pHOR30b	pBS plasmid containing <i>PEX19</i> deletion cassette; <i>URA3</i> ; <i>amp<sup>R</sup></i>	Otzen et al., 2004
pBS-CaLeu2	pBS plasmid containing <i>C. albicans</i> <i>LEU2</i> gene; <i>amp<sup>R</sup></i>	Otzen et al., 2004

Table S3. Primers used in this study

Primer	Sequence	Reference
Pex25-F	5'-CTGGATGGAGGCTTCATCTC-3'	Saraya et al., 2011
Pex25-R	5'-GGAGCTGCTGTGCTTGTATG-3'	Saraya et al., 2011
H5-F	5'-GTGCTGCAAGGCGATTAAGT-3'	This study
H5-R	5'-AGAGCTCGAGGTTAAGCATCGAAATTAGAGTAGAC-3'	This study
Pex13-F	5'-AAAAGCTTATGACTACACCACGTCCAAAGCC-3'	This study
Pex13-R	5'-AAAGATCTGATCAATAGCTTTTGATCTTTCTGAAC-3'	This study
Pex14-F	5'-CCCAAGCTTCGTTGCAGGAAGTCGACGAA-3'	This study
Pex14-R	5'-AGATCTTCCGGCATTGAGCTGCCACGCCG-3'	This study
Pex19-F	5'-AAAAGCTTATGAGCGAGAAAAAGTCC-3'	This study
Pex19-R	5'-ATCTAGACTATGTTTGTTGCAAGTGTCTTCC-3'	This study



Video 1. **Electron tomography of pex3 atg1 cells.** Two perpendicular tilt series ( $-70^\circ$  to  $70^\circ$ ) were acquired of 150-nm sections of  $\text{KMnO}_4$ -fixed cells with a transmission EM microscope (Tecnai 12; FEI) and reconstructed into a single tomographic volume by IMOD. Organelle masks were segmented manually in Amira followed by nonlinear anisotropic diffusion filtering, thresholding, and 3D surface rendering, using an identical color scheme as in Fig. 1 E.

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